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By M. I. Prokhorov,
Doctor of Veterinary Sciences

-USSR-

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THE THEORETICAL AND PRACTICAL BASIS OF DRY BACTERIAL CULTURES FOR THE CONTROL OF HARMFUL RODENTS.

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Rodent control is important in safeguarding agricultural harvests and food supplies, as well as in the prevention of various diseases.

The bacteriological method of controlling harmful rodents (discovered and developed by L. Pasteur, I. I. Mechnikov, N. F. Gamaleya, S. S. Merezhkovskiy, and B. L. Isachenko) has proved effective under different conditions, and, what is at least as important, it is safe for humans, animals and birds. This method is based on infecting rodents with bacteria lethal to them.

Observation of epizootic incidence in nature among rodents showed the presence of causative agents of the infectious diseases. These agents proved pathogenic to rodents, but harmless to humans, domestic animals and birds.

In our country, S. S. Merezhkovskiy (1893) isolated from the spermophile, bacteria pathogenic to various types of house and field mice. He named these Bact. typhi spermophilorum. Bacteria pathogenic to various types of rats, and house and field mice were isolated from rats by B. L. Isachenko in 1897, and named Bact. decumanicidum. In 1953 M. I. Prokhorov obtained experimentally bacteria No. 5170 for rat extermination.

When the bacteriological method is employed, house and field mouse mortality reaches 80% to 100%, while in rat extermination, mortality fluctuates from 60% to 100%.

After bacteria pathogenic to rodents had been discovered, research was directed toward finding cheap and ea ily obtainable nutrient media for their culture.

Many liquid media were proposed for preparing these bacterial cultures. S. S. Merezhkovskiy (1894) prepared bacterial cultures in plant broth, with the addition of molasses. For many years he used an alkaline meat broth (pH 7.2 - 7.4) in preparing bacterial

cultures for rat extermination purposes.

P. A. Bogdanov (1921) (3) began his research on milk media for culture growing, and obtained good results both in laboratory and in practical tests.

N. A. Dmitrievskaya and M. F. Chebotarevich, in 1929, (4) suggested the use of a brewer's yeast medium which had proved as effective as the meat broth and milk.

A. I. Antonovskiy (1) experimented with brewer's yeast media, and established that their bacteria titer can be higher than that of meat broth.

In 1942 M. V. Federov proposed media made of baker's yeast and peas. He showed that in these media the bacterial titer is no lower than in broth or milk, while the bacteria's pathogenic qualities do not diminish, but remain active for 45 days.

L. P. Krutikova (7) studied the pH, the titer, and the biochemical and pathogenic properties of the Isachenko bacteria on five different media (brewer's and baker's yeast, milk, peas and rye bran). She showed that media prepared from brewer's and baker's yeast, milk and peas are quite suitable for the preparation of Isachenko bacteria cultures. They retain their potency up to 45 days.

Various investigators have suggested media made from cabbage, fish and other products, but these media were not practicable.

Instructions for the preparation of bacterial cultures for rodent control recommend the tested and effective media made of peas, meat broth, milk, or brewer's or baker's yeast.

Liquid media cultures of the Isachenko and Merezhkovskiy bacteria are prepared by bacteriological laboratories and stations on order from organizations or households. However, after preparation, the bacteria titer of liquid cultures fluctuates between 0.4 and 0.7 billion bacteria per millileter.

Lethal infection of any type of house or field mouse requires between 0.4 and 0.6 (average 0.5) billion Merezhkovskiy or Isachenko bacteria. Consequently, the bait must contain 1 ml of the liquid culture.

The lethal infection of one rat (grey, black or water rat) requires from 5 to six (average 5) billion Isachenko or No 5170 bacteria. Conseuqently, the bait must contain 10 ml of the liquid culture.

With the liquid bacterial culture, the preparation of one bait for one domestic or field mouse requires from 1.8-2 grams of flour; for one rat, 18-20 grams.

The bacteria titer is relatively low in liquid media, while the lethal dose for a rodent is of large volume. For this reason, it was necessary to decrease the volume of the lethal dose, and, consequently, to decrease the amount of the product used in preparing the bait.

Liquid bacterial cultures are prepared locally, since they are awkward to transport long distances, and their potency lasts only 45-50 days.

In rodent control, it would be more convenient to use cultures whose bacteria titer is significantly higher, and whose potency would last longer periods of time. These requirements can be satisfied by dry cultures which would contain several billion bacteria in each gram.

Many research workers have studied the possibilities of obtaining dry microbe cultures; they have achieved positive results. Dry cultures of various microbe types can be obtained by first growing the bacteria on liquid media; this was shown by A. K. Konova and L. S. Bazilevskaya (6), I. L. Serbinova (10), S. S. Rechmenskiy (9), N. N. Titova (11), V. T. Bobovich (2), S. G. Kolesova (5), and others.

The small quantities of dry microbe cultures obtained by the various investigators had low titers, and were used primarily for laboratory experiments.

Achieving large quantities of dry bacterial cultures suitable for rodent control was related to the research on media which required an initial growing of the culture. Such dry or semi-dry media must have from 8% to 15% water. The dry fraction must contain the necessary nutrients (albumin, hydrocarbons etc.) in quantities sufficient for the maximal proliferation of microbes. At the same time, these nutrients must be easily accessible to the microbes.

The structure of dry or semi-dry media is no less important.

Just as the natural lumpy earth structure is optimal for greatest microbe proliferation, so the structure of organo-mineral granular fertilizers proved best for microbe multiplication in the earth, and for the increase in the earth's fecundity.

Nutrient media for the growth of microbes to be used in rodent control must have a particulate structure which combines a sufficient quantity of nutrients for microbe growth, with low water content. Humidity may be added in the process of transferring the inoculum to the medium.

In liquid media, maximal microbe development is related to the nutrients composing the medium, aeration, and other factors. The success of bacterial culture masses on solid media (e.g. meat-infused agar, gelatins, etc.) depends upon the size of the area seeded.

Granulated media composed of particles, present the optimal conditions for microbe proliferation when compared to liquid or solid media. It is well known that the surface area of all the particles contained in a given volume is much greater than the surface area of the volume itself. The surface area of the particles in the volume increases as the diameter of each granule decreases.

Consequently, the greatest advantage of the granular structure as compared to liquid and solid media is the enormous surface area of the medium's particles, on which a large mass of microbe colonies can develop. After infusion, the particles swell, and the porous structure of the particles permits microbes to penetrate the medium easily. This further increases their numbers in each volume or weight unit.

It is essential that the nutrient medium particles contain within themselves substances which produce colloidal solutions, and that they can swell quickly and easily after having been dampened with the infusion. After the microbe cultures have been grown, a rapid, and above all a complete dehydration must occur during the drying of such a medium.

Thus, dry media must combine the following attributes: low humidity, a sufficiency of nutrient substances (especially albumin), colloidal form of the particles, and the medium must be granular in structure. A combination of these properties, when correctly used, should lead to a maximal microbe multiplication. In rodent control, the use of cultures in dry media made from produce could make it possible either to decrease or to eliminate completely the bait, since the culture itself serves as bait.

Having found media which would answer all of these requirements, we then collaborated in research with L. Ya Sintsova and M. I. Shlyakhtenko on an animal-derived medium (fibrinous). Research on media of plant origin (from a variety of grains) was done together with V. T. Bobovich.

M. I. Shlyakhtenko derived a fibrinous dry medium from animals. This medium consists of separate particles whose diameter varies from 0.25-2 mm. The whole medium is a granulated mass, light-yellow in color, and preserves well at ordinary room conditions. The medium's humidity, both before and after sterilizing, usually remains at the same level -- from 8% to 10%. The medium has a neutral reaction. It is highly hygroscopic, and at the same time it easily loses humidity during drying. The medium has a complex composition in which the basic mass is made up of easily assimilated albumens.

When subjected to two sterilizations of from 20 minutes to two hours, at temperatures of 120°-127°, the animal-derived medium changes neither in its properties nor in its nutrient strength.

Only 24 hours later, it is possible to obtain on this medium 10 to 50 billion bacteria per gram of medium. After the bacterial culture has been dehydrated in a vacuum-drying apparatus, the bacteria titer decreases to 7-25 billion per gram.

With this medium, Isachenko and Merezhkovskiy bacteria, as well as the 5170 bacteria isolated by the Institute, are preserved in the dehydrated cultures for periods exceeding two years. The neutral reaction of the medium, and its characteristic biochemical, serological and pathogenic properties also remain stable.

Media of plant origin, obtained from various products, answered our requirements as well as did those derived from animal sources. In our investigations we employed different grits: buckwheat, barley, oats, peas, corn, as well as grains of rye and wheat which were ground until the particles were from 0.3-3 mm.

The composition of basic nutrient substances in the media is shown in the table.

The table shows that all the media contain a small amount of water. However, the amount is smallest in the animal-derived medium,

while the quantity of albumin is largest. After that follow the media made of peas, buckwheat and oat grits and the wheat grains. The highest quantity of fats is found in oat and corn grits, while wheat is highest in Hydro-carbons.

Sterilization experiments on dry media, both animal and plant based, showed that differing quantities of medium require different sterilization procedures. If the quantity of dry medium in a bottle or culture flask does not exceed 300 grams, then full sterilization is achieved by autoclaving

TABLE

Composition of Experimental Media's Components
(in percent)

	Type of medium	Water	Albumins	Fats	Hydrocarbons
1.	Animal-derived (fibrinous)	8--10	85,7	1,9	--
2.	Buckwheat grits	14	10,6	2,3	62
3.	Barley grits	13	6,6	0,8	67
4.	Oat grits	12	9	5	60
5.	Pea grits	13	16	1,6	50
6.	Corn grits	13	6,4	3,5	58
7.	Rye grains	12	8,6	1,5	60
8.	Wheat grains	12	9	0,9	70

at 120° for 20 minutes twice during the course of 48 hours.

When the volume of the vessel is enlarged, and consequently the quantity of medium as well, the temperature and the exposure time of sterilization increase.

Vessels containing from one to three kilograms of medium must be sterilized at 126° for two hours, twice during 48 hours. This sterilization procedure of dry media depends upon the air space between the particles of the medium. The thicker the air layer, the longer the period of sterilization. It is well known that air is a poor heat conductor. This is why, in order to heat all the particles of the medium situated at the center, higher temperatures and a longer heating period are necessary. Successful sterilizing of two to three kg. of dry media, especially in large autoclaves crowded with vessels, requires a pre-heating by steam for 30 minutes. The vessels are then heated for one hour at 1 atmosphere, and only after this is sterilization done. A gradual cooling of the vessels (from 40 to 50 minutes) is recommended after the end of sterilizing.

If initial pre-steaming of the autoclave, and heating of large volume vessels is not done, then it is possible that in some of them, especially toward the center, non-sterile particles will remain. During control seeding they can give growth to saprophytic microflora. However, if such medium-containing vessels are seeded with an infusion of bacterial culture used in rodent control immediately after cooling to 30°-37°, and are placed in an incubator, then optimal conditions

are created for the plentiful proliferation of bacteria which destroy saprophytic microflora. The saprophytes are either destroyed by lysis, or else their proliferation is so suppressed, that they could not be found even in repeated control seedings of undried prepared culture.

Live saprophytes, remaining after inadequate sterilizing will be fixed at the surface or in the depths of the particles. For this reason, these microbes cannot change their location as readily in a dry medium as in a liquid one.

Never once was a foreign microflora isolated in repeated bacteriological experiments with completed cultures grown in large volume vessels on solid media composed of particles.

In order to obtain a high bacteria titer when the infusion grown on MP (Bouillon), milk, yeast and other liquid media is seeded on to dry animal or plant derived media, a sufficiently thorough wetting of the particles of the dry medium is necessary; with this wetting, the particles absorb moisture and swell. Complete moistening of all the particles can be achieved by thorough mixing with a sterile spatula, or by shaking the entire mass of the medium after seeding. No excess moisture between the particles must remain. Such moisture would displace air, and would decrease aeration. This creates adverse conditions for the growth of aerobic cultures.

The optimal ratio of infusion and dry medium, animal or plant derived, is one part, by volume, of infusion to two weight parts of dry medium. With this ratio, the best bacteria multiplication occurs; bacteria titers reach high levels.

This ration of the infusion to the dry medium fosters a more rapid dehydration of the culture, and preserves a high titer after drying.

Research on the relation between the size of the particles of the medium on the one hand, and the intensity of proliferation and quantity of bacteria in the culture on the other, showed that particles of from 0.5-3 mm do not induce sharp fluctuations in bacteria titer. In media of plant origin in which the particles were from 0.5-3 mm, the titer fluctuated between 4-8 billion.

We experimented with dehydrating dry media cultures by using the vacuum-drying apparatus, VN 461-M, manufactured in the Soviet Union in 1950, category V, at the "Platinopribor" factory.

Dehydration was performed at temperatures ranging between 16°-68° for four to twelve hours.

During the course of the drying experiments, we established temperature and exposure conditions optimal for a minimal moisture residue (7%-10%-12%), and for a sufficiently high bacteria titer. Optimal drying conditions were those in which the drying temperature did not exceed 37°, and the duration of exposure was not less than 3.5-4 hours. During the entire drying period, the manometer gauge remained at the 760 mm level.

Directly after the cultures had been dried, the bacteria titer in one gram of medium (animal derived) was within the limits of

7-25 billion. At a later time after drying, the titer decreased by 10%-50% as compared to the initial.

After drying, and under the same conditions, the bacteria titer of cultures grown on plant derived media also fluctuated between 3-5 billion, i.e., it decreased by 10%-50%.

There is a gradual decrease in bacteria titer when the dried cultures are kept at ordinary aerial conditions in vessels sealed with wax.

Cultures grown on animal-derived media, for example, show a gradual decrease in the bacteria titer, and after 1.5 years it drops from the several billion of the initial titer to hundreds or tens of millions per gram.

After 2.5 years of storage, dried cultures grown on plant-derived media also exhibit a titer decrease down to hundreds and tens of millions. However, after a comparable storage period, we found one billion viable bacteria per gram in certain vessels with plant media.

High titers can be reestablished in dry cultures whose bacteria titer has decreased after prolonged storage. To do this, 24 hours before use, the cultures must be moistened with sterile water which had been heated to 37°. The proportions are: 1 part water (by volume) to 2 parts of dry culture (by weight). The culture must then be placed in an incubator at 37° for 24 hours. Following this procedure, the initial bacteria titer is reestablished, and such a culture, when mixed with bait or even without it, can be used successfully in rodent control.

It is important to note that after prolonged storage of dry cultures, the weak bacteria tend to die off, while the most viable ones, which are also the most pathogenic to rodents, survive.

Experiments showed that in dry cultures, viable bacteria keep their cultural, biochemical, serological and pathogenic properties, even after several years.

Experiments with undried media made of pounded rye and wheat showed that after storage the decrease in the initial titer of 6 to 7 billion bacteria is insignificant at temperatures of +5° and +10°; in 30 days of storage, it decreases to 4 billion; in 40 days, to 3 billion; in 50 days, to 2.1 billion; and in 60 days, the decrease is to 1.7 billion.

Consequently, bacteria cultures grown on media made of pounded rye and wheat, keep a bacteria titer of over two billion for 50 days, and only after 60 days does the titer drop below two billion. During this period, the neutral reaction of the medium remains constant. Thus it is possible to replace liquid bacteria cultures with cultures grown on plant media. They must be used up within 50 to 60 days after bacteria growth, and must be stored at temperatures ranging from +2° to +10°.

A higher storage temperature for undried cultures is not conducive to the maintenance of the bacteria titer.

Isachenko and No 5170 bacteria cultures, grown on dry media,

were tested for pathogenicity under laboratory and field conditions in rodent control.

Laboratory experiments indicated that all grey rats and field and house mice, after having been fed with freshly prepared or dried cultures, died within a period of seven to twelve days. Cultures having a bacteria titer of 4 billion and more, have the following potency: one gram of culture is necessary to infect and destroy one grey rat, while for house and field mice, 0.1 gram is sufficient. If the bacteria titer of the culture is lower than 4 billion, then the dose fed to the rodent must be increased.

Cultures grown on animal or plant derived dry media were tried in practical rodent control on certain farms in the Leningrad, Stalin, and Kherson districts, and in the Azerbaydzhanian SSR.

The experiments were conducted in forcing pits, on animal husbandry farms, graneries and vegetable storehouses, in the fields, pastures and in dwellings. Cultures grown on these media were used on fields and pastures exceeding 3,000 in area. In forcing pits, animal husbandry farms, in graneries and in dwellings the area exceeded 3000,000 square meters. The cultures were applied in a mixture with flour as bait. As the culture dose was decreased by five to ten times, the amount of bait used decreased correspondingly. No harmful effects were observed in humans, animals or birds in any of the households that used the bacteria cultures grown on the new dry media. There was only one case, on one of the kolkhozes in the Azerbaydzhanian SSR, where sparrows were destroyed.

Thus the new media, both animal and plant derived, proved quite useful for growing bacteria specific to rodent control.

These media permit the growth of bacteria having from 10 to 100 times higher titers than liquid media. After dehydration the culture mass increases ten times.

High bacteria titers in dry media made from animal or plant bases were obtained as a result of the medium's granular structure. In such media, microbes proliferate not only on the large surface area of the nutrient particles, but also inside the particles, after they have swollen from the moisture introduced with the inoculum.

Dry media proved to have a sufficient quantity of nutrient substances. They do not need the various addatives usually necessary in the liquid media from which dry cultures are derived. It was established that the granular particles in dry media are very hygroscopic, and at the same time lose water easily. Because of this, microbe proliferation occurs rapidly on their surface, and afterwards, it is easy to dry them to the minimal residual moisture.

Dry microbiological media may be useful in making various other bacterial preparations applicable to agricultural needs, as for instance, bacterial fertilization. They could also be useful in preparing vaccines for medical and veterinary practice.

CONCLUSIONS

1. Dry cultures with high titer and in large quantities cannot be derived from liquid bacterial cultures used in rodent

control.

2. Dry media made of animal and plant material, with a granular structure, have been discovered. Large quantities of dry culture with a high bacteria titer per gram have been grown on the particle surfaces.

3. Dry media of plant origin were obtained from grits: buckwheat, oats, barley, corn and peas, as well as from grains of rye and wheat.

4. An important advantage of microbiological nutrient media with a granular structure over solid and liquid media is the enormous surface area of the granules on which large quantities of bacteria can develop.

5. Large quantities of microbes are obtained on dry media because of their high nutrient value, their granular structure, and the colloidal state of the particles.

6. The following strains have been grown on dry media: Isachenko's bacteria (Bact. decumanicidum), Merezhkovskiy's bacteria (Bact. typhi spermophilorum), and the new No 5170 bacteria isolated for rodent control by the Institute of Agricultural Microbiology.

7. Bacteria grown on dry media and dehydrated in a vacuum-dryer apparatus, keep their cultural, biochemical, serological, and pathogenic properties for periods exceeding $2\frac{1}{2}$ years.

8. Bacterial cultures applicable to rodent control, when grown on media of plant origin, can, without being dried, retain a high titer for more than 60 days if kept at temperatures of 5° to 10°.

9. The use of bacterial cultures grown on dry media for rodent control has shown effective results as well as the important advantages of such media. These advantages are: the weight of a lethal culture dose decreases from five to ten fold; expenditure of the product used as bait decreases from five to ten fold; the work involved is greatly reduced, and the culture's transportability is increased.

10. It would be desireable if dry media of plant origin were used in inter-district and area bacteriological laboratories in their preparation of bacterial cultures for rodent control.

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